REMARKS

Claims 26-41 have been canceled. Calims 42-67 have been added. . Support for the new claims can be found throughout the specification including the Drawings and claims as filed originally. No new matter has been added. Applicant appreciates the Examiner's acknowledgement of Applicant's claim for foreign priority based on an application filed in Germany on January 23, 2001. A certified copy of the 10102977.2 application will be submitted under separate cover as soon as it is received from the German Patent Office.

Applicant now turns to comments made by the Examiner in the Office Action as follows.

1. The listing of references in the specification is not a proper information disclosure statement.

> The Examiner states "37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered. The specification on pages 15-24 comprises a list of references. If these references are to be considered, they must be listed on an IDS and copies of the references must be submitted.".

There is only one reference cited on pages 15-24 of the specification that has not been listed in a previously filed Information Disclosure Statement. Applicant will submit an IDS on form PTO-892 listing that reference.

2. Claims 32-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

> The Examiner states "Claims 32-37 provides for the use of a cell line, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced.".

Claims 32-37 have been rejected as being indefinite because of a lack of recitation of specific method steps involved in the claimed 'use'. This has been remedied by the amended claim language in the new claims 59-64.

3. Claims 26, 29, 30, 38-40 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility.

> The Examiner states, "When determining whether an applicant has described the utility of an invention, one has to determine whether the applicant has described a well-established utility. If not, has the application made any assertion of specific, substantial, and credible utility. A credible utility is assessed from the standpoint of whether a person of ordinary skill in the art would accept that the recited or disclosed invention is currently available for use. In contrast to general utility, a specific utility will be specific to the claimed subject matter. A substantial utility defines a "real world" utility of the invention and utilities that require or constitute carrying out further identify or reasonably confirm a real world context use are not substantial utility (see utility guidelines, in Federal Register, January 5, 2001, Volume 66, Number 5, pages 1092-1099).
>
> When the claims are analyzed in light of the specification, the instant invention encompasses:
>
> a cell line comprising a loss of expression or reduction of expression of CICI-4,

a cell line that expresses CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7

a cell line comprising a loss of expression or reduction of expression of CIC-6,

and

a cell line that expresses CIC-6, but not CIC-3, CIC-4, CIC-5, and CIC-7.

In order for one to analyze utility of the claimed invention, it is asked if the proteins, CIC-4 and CIC-6, have an established and specific biological function.

With regards to the instant invention encompassing a cell line comprising a loss of expression or reduction of expression of CIC-4 and a cell line that expresses CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7, neither cell line is considered to have a specific and substantial utility. At the time of filing, the art teaches that CIC-4 has a sequence similar to CIC-3 and CIC-5 (Jentsch, et al., 1999, Pflugers Arch—Eur. J. Physiology, 437: 783-795; page 789, 1st col, lines 3-4) and the art teaches that stably transfected CHO cells expressing hCIC-4sk demonstrated a rectifying current when the pH was changed from 7.2 to 4.5 (Kawasaki et al. 1999, Am. J. Physiol., (Cell Physiol. 46) 277:

C948-C954). However, neither the specification nor the art teach what the biological function of CIC-4 is, (i.e. what role it has in the organism). It is emphasized that the CHO cell assay, which demonstrated a rectifying current does not demonstrate a specific activity of CIC-4. Rather, it is a general assay for chloride channels and cannot be used to distinguish chloride channels from each other. As such, the art teaches that the chloride family is divergent and the channels perform different functions. These functions include the control of electrical excitability, transepithelial transport, and the charge compensation necessary for the acidification of intracellular organelles. addition, CLC channels may play a role in cell volume regulation (Jentsch, et al., 1999, Pflugers Arch—Eur. J. Physiology, 437: 783-795; page 783, 2nd col., 2nd parag., lines 6). Nothing in the specification indicates that CIC-4 has any of these functions, or any newly identified function. Therefore, the specification does not teach that CIC-4 has a biological activity, explicitly or implicitly as considered by the specification. There is no teaching of any assay method for assaying the function of CIC-4. It is emphasized that the CLC family is diverse (Jentsch, et al., 1999, Pflugers Arch-Eur. J. Physiology, 437: 783-795 demonstrates a dendrogram indicating

the degree of similarity between different CLC gene products, Figure 1) and the biological functions of the CLC family are diverse. In other words, the only immediate apparent utility for the instant invention would be its further scientific characterization as a putative CLC. Finally, the specification does not provide any evidence as to what diseases the claimed polynucleotide or encoded protein is related to. In the absence of such a disclosure, it is unclear what diseases could be treated with the candidate thereapeutic compounds identified by the claimed method and if no such relationship of a disease with the protein of the claimed invention is known what will be the utility of the compounds.

Therefore, the asserted use for the claimed cells is not considered to be supported by either a specific and substantial utility since no function can be ascribed to CIC-4.

With regards to the instant invention encompassing a cell line comprising a loss of expression or reduction of expression of CIC-6, and a cell line that expresses CIC-6, but not CIC-3, CIC-4, CIC-5, and CIC-7, neither cell line is considered to have a specific and substantial utility. While the art teaches that CIC-6 has the structure of a chloride channel (Brandt and Jentsch, 1995, FEBS Letters, 377: 15-20, page 18, 2nd col., 3rd parag., see also Figure 2) and electrophysiology studies demonstrated that CIC-6, when expressed in Xenopus oocytes, has a outwardly-rectifying current and inactivated slowly at positive potentials (Buyse, et al., 1997, JBC, 272: 3615-3621, page 3618, 1st col., 2nd parag., lines 1-5), neither the specification nor the art teach what the biological function of CIC-6 is, (i.e. what role it has in the organism). It is emphasized that the Xenopus oocyte assay, which demonstrated a rectifying current does not demonstrate a specific activity of CIC-6. Rather, it is a general assay for chloride channels and cannot be used to distinguish chloride channels from each other. As such, the art teaches that the chloride family is divergent and the channels perform different functions. These functions include the control of electrical excitability, transepithelial transport, and the charge compensation necessary for the acidification of intracellular organelles. In addition, CLC channels may play a role in cell volume regulation (Jentsch, et al., 1999, Pflugers Arch—Eur. J. Physiology, 437: 783-795; page 783, 2nd col., 2nd parag., lines 1-6). Nothing in the specification indicates that CIC-6 has any of these functions, or any newly identified function. Therefore, the specification does not teach that CIC-6 has a biological activity, explicitly or implicitly as considered by the specification. There is no teaching of any assay method for assaying the function of CIC-6. It is emphasized that the CLC family is diverse (Jentsch, et al.,

> 1999, Pflugers Arch—Eur. J. Physiology, 437: 783-795 demonstrates a dendrogram indicating the degree of similarity between different CLC gene products, Figure 1) and the biological functions of the CLC family are diverse. In other words, the only immediate apparent utility for the instant invention would be its further scientific characterization as a putative CLC. Finally, the specification does not provide any evidence as to what diseases the claimed polynucleotide or encoded protein is related to. In the absence of such a disclosure, it is unclear what diseases could be treated with the candidate therapeutic compounds identified by the claimed method and if no such relationship of a disease with the protein of the claimed invention is known what will be the utility of the compounds.
>
> Therefore, the asserted use for the claimed cell is not considered to be supported by either a specific and substantial utility since no function can be ascribed to CIC-6.'.

Claims 26, 29, 30 and 38-40 have been rejected as lacking a disclosed substantial asserted utility or a well established utility. Claim 26 has been deleted without replacement, thereby obviating its rejection.

Claim 29 was directed to a cell line which expresses ClC-4 but not certain other cell lines and the rationale for the objection is that there is no disclosed biological function for ClC-4. It is therefore alleged that it is unclear what diseases could be treated with a therapeutic compound which comprises ClC-4.

With respect to the Examiner, we submit that the objection overlooks the utility of the relevant cell line (and the corresponding cells, cell membrane preparations and cell vesicles). The purpose envisaged for making a cell line that expresses ClC-4 but not certain other ClC chloride channels is not necessarily to provide ClC-4 for a therapeutic use. Neither is it necessarily to find compounds that are inhibitors or other modulators of ClC-4.

The inventors' intention is primarily to find modulators of the ClC-7 chloride channel and preferably these should be specific modulators of that chloride channel and should have more limited effect on other chloride channels.

Cells expressing ClC-4 but not ClC-7 are useful in this task. Compounds may be identified (by other means) that seem to modulate ClC-7 in ClC-7 expressing cells, but it will be useful to exclude the possibility that the measured effects of such compounds are due not to modulation of ClC-7 as desired but rather to modulation of ClC-4, expressed in the same cells. The $ClC\text{-}4^{+}/ClC\text{-}7^{-}$ cells claimed previously in claim 29 and now in claim 51 will be useful as a second screen for such compounds. Those that show no modulating activity when tested against such a cell line are better candidates for being specific ClC-7 modulators.

To put it another way, the use of the cell line of claim 29 to find modulators of ClC-4 may serve to identify such compounds not because they are wanted but because one wants to exclude them from further study as specific inhibitors of some other chloride channel. Such a utility is described in the specification on page 7, lines 5 onwards.

The Examiner's conclusion on page 7, i.e. that 'the specification does not provide any evidence as to what diseases *the claimed polynucleotide or encoded protein* is related to' seems to have no direct bearing on claim 29, which is not directed to any polynucleotide or encoded protein.

The cell lines which were covered by claim 29 on the other hand have a clear utility in screening for say ClC-7 specific inhibitors (as a means of excluding non-specific ClC-7 inhibitors) as disclosed in the application.

We respectfully submit therefore that the objection should be withdrawn.

Exactly similar arguments apply in relation to the objection under 35 USC 112 to claim 30 (now replaced by claim 54), the cell lines of which are useful at least for screening out compounds that are not specific for ClC-7 but which act on ClC-6.

Similar arguments apply also in relation to previous claims 38-40 and their rejection under 35 USC 112. It is useful to identify substances which inhibit say ClC-4, if only to exclude them as specific ClC-7 inhibitors.

4. Claims 26-30, 32-40 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

a cell line comprising a disruption in its endogenous CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-5, and/or CIC-7 gene of the mouse genome, wherein in there is no expression of functional CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-5, and/or CIC-7 from the endogenous gene,

does not reasonably provide enablement for:

a cell line comprising a loss of expression or reduction of expression of CICI-4,

a cell line that expresses CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7

a cell line comprising a loss of expression or reduction of expression of CIC-6,

and

a cell line that expresses CIC-6, but not CIC-3, CIC-4, CIC-5, and CIC-7.

> The Examiner states, "The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.
>
> Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as

routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " *(Wands, 8* USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." *(Wands, 8* USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case are discussed below.

In addition to the issues raised in the utility rejection regarding CIC-4 and CIC-6, the claimed invention is not enabled for the reasons set forth below. If the utility rejection was to be withdrawn, these rejections would remain.

When the claims regarding CIC-4 and CIC-6 are analyzed in light of the specification, the instant invention encompasses

a cell line comprising a loss of expression or reduction of expression of CICI-4,

a cell line that expresses CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7

a cell line comprising a loss of expression or reduction of expression of CIC-6, and

a cell line that expresses CIC-6, but not CIC-3, CIC-4, CIC-5, and CIC-7,

and methods of using said cell lines in a screen to identify substances which are suitable for inhibiting one or more chloride channels, including CIC-4 and CIC-6. As discussed in the utility rejection, there is no guidance and evidence in the specification as to what the biological function is of CIC-4 and CIC-6, how to test the biological functions of CIC-4 and CIC-6, which disease the protein's expression is related to, and how to screen for compounds that inhibit CIC-4 and CIC-6. The art teaches that the CLC family of proteins is diverse and the family members perform different biological functions (Jentsch, et al., 1999, Pflugers Arch—Eur. J. Physiology, 437: 783-795, see page 783, 2nd col., 2nd parag., lines 1-

6 and Figure 1). Additionally, the art does not teach which disease(s) CIC-4 and CIC-6 is associated with. Thus, it stands that an artisan has to establish the relationships between CIC-4 and a disease and CIC-6 and a disease before cells comprising expression of CIC-4, but not CIC-3 CIC-5, CIC-6, CIC7 and cells comprising expression of CIC-6, but not CIC-3, CIC-4, CIC-5, CIC-7 can be used in a screen for therapeutics.

With regards to using the cells comprising expression of CIC-4, but not CIC-3, CIC-5, CIC-6, CIC7 and cells comprising expression of CIC-6, but not CIC-3, CIC-4, CIC-5, CIC-7 in a method for identifying substances which inhibit CIC-4 and CIC-6, the specification as filed does not teach any assays that monitor the biological function of CIC-4 or CIC-6. As such, an artisan would not know how to screen for compounds if no guidance is taught as to what biological function is monitored.

Therefore, an artisan would have to carry out extensive experimentation to first characterize CIC-4 and CIC-6 to determine its relationship to a biological function, establish an assay system to detect the activity of CIC-4 and CIC-6, and determine a relationship of the CIC-4 and CIC-6 to a phenotype before it can be used for screening of candidate therapeutic compounds. Such experimentation would be undue since neither the art nor the specification provides any guidance.

Accordingly, in view of the quantity of experimentation necessary to establish a biological function of CIC-4 and CIC-6, to establish its relationship to a disease, and to establish an assay system to determine its biological activity, the lack of direction or guidance provided by the specification, and the absence of working examples with regards to the method claimed, it would have required undue experimentation of one skilled in the art to use the claimed invention.

In addition to the enablement issues addressed for cells comprising CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7 and cells comprising CIC6 but not CIC-3, CIC-4, CIC-5, and CIC-7, other enablement issues are directed to claim 26, which broadly claims "a cell line which does not express or express only to a reduced extent one or more chloride channels from the group consisting of CIC-1, CIC-2, CIC-1, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, and CIC-7." Claims 27-30 depend on claim 26.

With regards to a cell line which does not express or expresses to only a reduced extent one or more chloride channels, this encompasses cell lines that express RNAi, ribozymes, and antisense RNA. At the time of filing, the specification does not

teach an artisan how to make RNAi, ribozymes, and antisense which specifically target chloride channels, thereby reducing their expression. At the time of filing, the use of RNA molecules such as RNAi, ribozymes, and antisense RNA to predictably and routinely effect a biological change is not known in the art. Rather there have been many examples where the introduction of RNA molecules for purposes of gene therapy have resulted in unexpected results. For example, a review by Agrawal and Kanimalla (2000, Molecular Medicine Today, 61: 72-81) teaches that the use of antisense as a means of targeting a gene is unpredictable. "A good part of nucleotide design for its target RNA varies significantly, depending on base composition and sequence. Therefore, the antisense activity of a selected oligonucleotide is influenced both by its base composition and by its sequence. Introduction of oligonuclotides that contain certain sequence motifs, such as CpG and GGGG (hyper-structure-forming sequences) induce cell proliferation and immune responses.... If an antisense oligonuclotide possess self-complementarity or a palindromic sequence, it can form stable secondary structures such as short linear duplexes or hairpins. In such cases, secondary structure formation competes for binding to the target mRNA. In addition, these secondary structures can serve as decoys by binding to cellular factors, thereby inhibiting or inducing the functions of non-targeted genes, which could directly or indirectly alter the function of the gene being studied (page 77, second column, "Choice of oligonucleotide sequences," first and second paragraphs)." Thus, because not all antisense constructs will function as predicted, all antisense constructs need to be tested for function and efficacy. The specification does not provide any examples of antisense constructs that were able to reduce the expression level of a target gene and thus does not enable one skilled in the art to make and/or use a plasmid vector expressing therapeutic RNA in the treatment of an animal. While Agrawal and Kanimalla teach that designing antisense molecules are unpredictable as described above, it should be pointed out that for similar reasons, the making of RNAi and ribozymes are unpredictable as well.

With regards to a cell line which does not express or expresses to only a reduced extent one or more chloride channels, this encompasses cell lines in which the regulatory regions of chloride channels are altered such that the expression of mRNA encoding a chloride channel is reduced or absent from a cell. At the time of filing, the art teaches that identifying regulatory regions in promoters was unpredictable. For example, Goswami et al.

(2003, Journal of Molecular Evolution, 57:44-51) teach some of the analyses used to characterize a promoter. Goswami et al. show by 5' deletion analysis that BD2, a greater 5' deletion of the TGF-f35 promoter than BD3, has more activity than BD3, suggesting that the 5' deletion in BD2 uncovered a negative regulator in the promoter (page 46, column 2, first paragraph, lines 3-7). Goswami et al. also show that while there is this difference in promoter activity between the two constructs transfected in XTC cells *(Xenopus* tadpole cell line), there is no difference in the activity of the promoters when transfected in A6 cells *(Xenopus* adult kidney fibroblast cell line). This result suggests that there is a difference in the transcriptional factors between the cell types (page 46, column 2, first paragraph, lines 7-10). Goswami et al. also show that there is a difference in promoter regulation, depending what animal species that promoter is from and into which cells the reporter construct is transfected. TGF-β5, which is found in rats and frogs, was found to be regulated differently. *Xenopus* TGF-β5 transfected into *Xenopus* cells had activity; it had little to no activity when transfected into mammalian cells (page 47, column 2, section headed "Basal Promoter Activites of TGF-β5 and TGF-β5 Promoter in Mammalian Cell Lines", see also Figures 3 and 4). In applying the teachings of Goswami et at. to the instant application, the specification does not teach how to predictably obtain a promoter that regulates a chloride channel's mRNA expression and alter it such that the expression driven from the promoter is less than what it was originally. The specification does not teach what regulators comprise any of the chloride channels' promoters (i.e. negative or positive regulators), such that deletion of the positive regulators results in downregulation of gene expression. As such because identifying where and what the regulators are in a promoter is unpredictable and is done empirically, obtaining any chloride channel promoter without guidance on how to alter it to express little to no chloride channel transcript would be undue experimentation.

Claims 32-40 are to methods for identifying and testing of substances which inhibit CIC-7, CIC-3, CIC-4, and CIC-6, wherein identifying and testing is carried out on the claimed cells. As described above, for reasons of utility and enablement, an artisan does not know how to use any cell comprising CIC-4 and any cell comprising CIC-6, in a method for testing and identifying a substance that inhibits CIC-4 or CIC-6. Thus, claims 35, 36, and 37 (as it reads on CIC-4 and CIC-6) will be excluded from this part, of the analysis regarding a method of testing and identifying a compound.

The art and the specification enables an artisan to use an osteoclast cell line which expresses CIC-7, but not CIC-3,CIC-4, CIC-5, and CIC-6 and a hippocampal cell line which expresses CIC-3, but not CIC-4, CIC-5, CIC-6, and CIC-7. With regards to claim 33, the Examiner has interpreted the claim to read that the cell used in a method for identifying and testing substances that treat osteoporosis of Paget's disease, is an osteoclast cell line which expresses CIC-7, but not CIC-3, CIC-4, CIC-5, treated with a substance that inhibits CIC-7. If this is the case, then the osteoclasts have little to no active CIC-7 and would behave in a manner similar to osteoclasts in knockout CIC-7 mice. The knockout CIC-7 mice exhibit osteopetrosis (thickened sclerotic bones, resulting from deposition of bone and reduced loss of bone) and not osteoporosis (bone loss) or Paget's disease (a disease wherein the bone breaks down more quickly than grows). Osteoporosis and Paget's disease, symptom-wise, are opposite to osteopetrosis. Nothing in the art or the specification teaches how to use an osteoclas cell line which expresses CIC-7, but not CIC-3, CIC-4, CIC-5 to screen for substances which could alleviate bone loss. Without guidance as to how an artisan would perform this screen is undue experimentation.

With regards to claim 37, wherein an osteoclast cell line which expresses CIC-7, but not CIC-3, CIC-4, CIC-5, and CIC-6 and a hippocampal cell line which expresses CIC-3, but not CIC-4, CIC-5, CIC-6, and CIC-7, is used to screen for psychotropic pharmaceuticals, nothing in the art of the specification teaches that an osteoclast has a role in controlling emotions. In addition to this, nothing in the art or the specification teaches that CIC-3 has a role in an area of the brain that controls emotions. With regards to whether a hippocampal cell line which expresses CIC-3, but not CIC-4, CIC-5, CIC-6, and CIC-7 can be used in a method to screen for compounds suitable for psychotropic pharmaceuticals, the art teaches that depression can result from glutamate-induced exocitotoxicity in the hippocampus. For this reason, the specification and the art enable an artisan to practice a method of screening for a psychotropic pharmaceutical using a hippocampal cell line which expresses CIC-3, but not CIC-4, CIC-5, CIC-6, and CIC-7.

Thus, the specification, while being enabling for:

a cell line comprising a disruption in its endogenous CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-5, and/or CIC-7 gene of the mouse genome, wherein in there is no expression of functional CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-5, and/or CIC-7 from the endogenous gene,

does not reasonably provide enablement for:

a cell line comprising a loss of expression or reduction of expression of CICI-4,

a cell line that expresses CIC-4, but not CIC-3, CIC-6, CIC-5, and CIC-7

a cell line comprising a loss of expression or reduction of expression of CIC-6,

and

a cell line that expresses CIC-6, but not CIC-3, CIC-4, CIC-5, and CIC-7.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 26-30 and 32-40 have been rejected as lacking enablement in respect of cell lines lacking effective expression of ClC-4 or ClC-6.

On page 12 of the office action it is alleged: 'Thus, it stands that an artisan has to establish the relationships between ClC-4 and a disease and ClC-6 and a disease before cells comprising expression of ClC-4 but not ..other ClC's..and cells comprising expression of ClC-6, but not ...other ClC's...can be used as a screen in therapeutics.'

Applicant respectfully points out that whether this is true or not (and as explained above, we say 'not'), this is not a basis for a rejection for lack of enablement in relation to the claimed cell lines. It goes purely to their usefulness and is not a basis for alleging that they cannot be made.

This same point can be made against all of the Examiner's arguments in relation to enablement of cell lines extending up to the beginning of the last paragraph of page 13. In short, no knowledge of the biological function of ClC-4 or ClC-6 or any other ClC channel is needed to produce the claimed cell lines.

Starting with the final paragraph on page 13, the Examiner begins to raise a genuine objection of lack of enablement against these claims by presenting reasons why allegedly a skilled person could not make such cell lines using certain techniques based on expression of RNAi, ribozymes and antisense RNA. This objection runs to the start of the last paragraph on page 16.

Nowhere in the discussion does the Examiner discuss the methods actually taught in the specification for making the desired cell lines. The objection does not seem to be that the Applicant has not provided an enabling disclosure of how to make cell lines as claimed, but rather that there are still other methods known in principle in the art that might not work without undue experimentation. The Examiner seemingly accepts that the methods based on homologous recombination described in the application work without undue experimentation. This is apparent from the rejections for lack of novelty and non-obviousness to which we shall be addressing in due course.

It is in our submission sufficient for an applicant to describe how a claimed invention can be made. There is in our submission no obligation on an applicant to describe how to succeed also using methods that may be more problematic and less desirable than those disclosed.

Commencing with the final paragraph on page 16, it is objected against claims 32-40 that the skilled person does not know how to test for compounds which inhibit ClC-7 and other chloride channels using the claimed cell lines. Screening for inhibition of ClC-4 and ClC-6 is particularly objected to, to the extent that such screening is excluded from the remainder of the Examiner's analysis.

We believe that we have already demonstrated above in relation to the utility of cell lines as claimed in screening methods that there is no lack of teaching of how to make use of such cell lines, nor of any teaching of how to make the cell lines for use. In short, relevant cell lines can be made by known techniques of homologous recombination and they can be used to screen compounds to determine the degree to which they are ClC-7 specific modulators by seeing whether they also modulate ClC-4 or ClC-6 and no knowledge of the biological function of either ClC-4 or ClC-6 is needed for any part of this exercise.

In the first full paragraph of page 17, a different rejection for lack of enablement is raised. The argument runs in relation to claim 33 that a ClC-7 expressing cell which is deficient in other ClC channels is treated with candidate substances, some of which will inhibit the ClC-7 channel. When that happens, the osteoclasts will behave as in knockout ClC-7 mice, which exhibit osteopetrosis due to a reduction of the rate of bone loss. From this, the Examiner perceives some difficulty in the screen being useful for compounds that will treat osteoporosis or Paget's disease, which are characterised by excessive bone resorption.

We respectfully regret that we are unable to follow the logic of the objection. A compound that will successfully treat osteoporosis or Paget's disease will be one that will counter the excessive bone loss that characterises the disease. This can be done in principle by either increasing the rate of bone formation or by reducing the rate of bone loss. That is exactly what the Examiner correctly states will be the effect of a compound that inhibits ClC-7.

Inhibition of ClC-7 will reduce the activity of the osteoclasts and hence will treat diseases characterised by excessive osteoclast activity causing a net loss of bone mass.

Rejection has been made to claim 37 in which an osteoclast cell line expressing ClC-7 but not some other chloride channels is used to screen candidates for psychotropic pharmaceuticals. Strictly speaking claim 37 was not limited to the cells being osteoclasts or hippocampal cells and the method in question clearly does not depend on osteoclasts having any role in controlling emotions.

The theoretical basis underlying claim 37 is set out in the specification on page 2 starting at line 7. ClC-3 is relevant to synaptic transmission. The cell lines in question are suitable for use in positive or negative screens for specific modulators of ClC-3. Compounds modulating ClC-3 will be expected to affect synaptic transmission and are therefore candidates as psychotropic pharmaceuticals. The nature of the cell is not directly material here. The cell is merely a tool for conveniently allowing the action of the chloride channel to be observed under the influence of the test compound. Accordingly, the method is just as well enabled using osteoclasts as it is with hippocampal cells. Furthermore, because negative screens are useful in identifying selective inhibitors of ClC-3, cells which effectively express say ClC-7 but not ClC-3 are useful in such screening assays. They allow previously identified ClC-3 modulators that are not sufficiently selective to be excluded.

5. Claim 26 is to a cell line which does not express or expresses to only a reduced extent one or more chloride channels from the group consisting of CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, CIC-7.

> The Examiner states, "Claims 26 is rejected under 35 U.S.C. 102(b) as being anticipated by Matsumura et al. (1999, Nature Genetics, 21: 95-98, see IDS).
>
> Matsumura et al. teach that Clcnkl-/- (wherein the human homolog is CIC-Ka) mice were generated using homologous recombination in embryonic stem cells (Matsumura et al., page 95,

2nd parag. 1st parag. following the Abstract). Embryonic stem cells are used to generate knockout mice are obtained from cell lines. Thus, Matsumura et al. anticipate claim 26.

Claim 26 is rejected under 35 U.S.C. 102(a) as being anticipated by Piwon et al (2000, Nature, 408: 369-373, see IDS).

Piwon et al. teach that CIC-5-/- mice were generated using homologous recombination in embryonic stem cells (Piwon et al., page 373, under "Generation of clcn5- mice"). Embryonic stem cells are used to generate knockout mice are obtained from ES cell lines. Thus, Piwon et al. anticipate claim 26.

Claim 26 is rejected under 35 U.S.C. 102(b) as being anticipated by Lupo et al. (1997, JBC, 272: 31641-31647).

Lupo et al. teach that NIH3T3 cells were treated with actinomycin D, a specific inhibitor of RNA polymerase II (Lupo et al., page 31643, 1st col., 1st parag. under "Effects of Actinomycin D and Cycloheximide on Aldolase A L- and F-type mRNA Expression," lines 1-6). Treatment of the cell line NIH3T3 with actinomycin D inhibits RNA polymerase II. This means that transcription for all genes is inhibited when cells are treated with actinomycin D. This includes transcription of the chloride channels. Thus, Lupo et al. anticipate claim 26.".

Only claim 26 was rejected under this heading and claim 26 has been canceled without replacement, thus obviating the rejection.

6. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gronemeir et al. (1994, JBC, 269: 5963-5967, see IDS) in view of Capecchi (1989, Science, 244: 1288-1292) and Alberts et al.(1994, Molecular Biology of the Cell,

Garland Publishing: New York, 3rd ed.).

The Examiner states, "Gronemeier et al. teach that the ADR mouse is a model for human myotonia. The adr allele arose by insertion of a retroposon into the chloride channel gene CIC-1 (Gronemeier, et al., Abstract, lines 8-10). Gronemeier et al. also teach that the chloride channel CIC-1 is necessary for the stabilization of the resting potential in mature vertebrate muscle (Gronemeier, et at, Abstract, lines 1-3).

While Gronemeier et al. teach a mouse comprising a disruption in CIC-1, they do not teach a cell line comprising a disruption in CIC-1.

With regards as to why an ordinary artisan would want to obtain a cell line, Capecchi teaches that one advantage to carrying out in vitro studies over in vivo studies is that many biological questions can be answered directly and more simply with tissue culture systems (Capecchi, page 1288, 3rd parag., lines 3-4).

The generation of a cell line is well known in the art. For example, Alberts et at. teaches that wile most vertebrate cells die after a finite number of divisions in culture, some cells in culture will undergo a genetic change that makes them effectively immortal. Such cells will proliferate indefinitely and can be propagated as a cell line (Alberts et at. page 160, 1st parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells"). Alberts also teaches that cell lines can be propagated fro cancer cells. Cancer cells differ from those prepared from normal cells. For example, cancer cell lines often grown without attaching to a surface and they proliferate to a very much higher density in a culture dish. Similar properties can be experimentally induced in normal cells by transforming them with a tumor-inducing or chemical (Alberts et al., page 160, 2nd parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells").

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to generate a cell line from an ADR mouse comprising a disruption in CIC-1.

One having ordinary skill in the art would have been motivated to generate a cell line from an ADR mouse in order to use the cell in a screen that identify substances that rescue a cell from the phenotypes caused by CIC-1 disruption. Capecchi teaches that many biological questions can be answered directly and more simply with tissue culture systems.

There would have been a reasonable expectation of success given Gronemeier et al for teaching an ADR mouse comprising a disruption in CIC-1, wherein the mouse exhibits a muscle phenotype and Alberts et al. for teaching how to obtain a cell line from a primary culture.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.'.

Only claim 26 was rejected under this heading and claim 26 has been canceled without replacement, thus obviating the rejection.

7. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Blaisdell et al., (1999, Am. J. Respir. Cell Mol. Biol., 20: 842-847) in view of Chalaka et al. (1999, Am. J. Physiol., 277 (Lung Cell. Mol. Physiol. 21): L197-L203) and Capecchi (1989, TIG, 5: 70-76).

> The Examiner states, "Blaisdell et al. teach that when primary fetal distal lung epithelial cells were treated with keratinocyte growth factor (KGF), the mRNA and protein expression of CIC-2 increased in the cells (Blaisdell, page 845, 2nd col., 1st parag. under "Discussion"). Blaisdell et al. teach that KGF leads to fluid accumulation in fetal mouse lung explants and that the fluid expansion of the developing lung occurs by accumulation of chloride and water in the airways (Blaisdell, et al., page 846, 1st col., 1st parag., lines 1-3). Blaisdell et al. teach that non-CFTR chloride secretion exists in the developing fetal lungs because CFTR is not essential for normal lung morphogeneisis (Blaisdell, page 826, 1st col., 2nd parag., lines 1-3). Blaisdell et al. teach that KGF expression in the fetal lung epithelium contributes to fetal lung fluid expansion. Blaisdell et al. teach that aberrant signaling of KGF or an altered regulation of CIC-2 could result in congential malformations of the lung in newborns by affecting pulmonary fluid distension (Blaisdell et al., page 846, 2nd col., 3rd parag., lines 5-12)
>
> While Blaisdell et al. teach a that CIC-2 protein and mRNA expression increases in lung epithelial cells when induced with KGF, they do not teach a fetal lung epithelial cell line comprising a disruption in CIC-2.
>
> A fetal lung cell line is well known in the art. Chalaka et al. teach that a rat pre-type II cell line, FD18 was obtained from rat fetal lungs at gestational days 18-19 and immortalized with the adenoviral 12S E1A gene product (Chalaka et al., page L197, 1st col., 1st parag. under "Material and Methods, Cell Culture").
>
> Capecchi teaches a method wherein targeted disruption of a gene of interest can be achieved via homologous recombination. Capecchi teaches the parameters used to increase an artisan's chances of performing homologous recombination, which includes, "the frequency of recombination between co-introduced DNA molecules is roughly proportional to the extent of homology

between them. When DNA molecules share more than 5 kilobases of homology, then nearly every molecule introduced into the cell nucleus participates in at least one recombination event....DNA ends stimulate the reaction. The ability to mediate homologous recombination is dependent on the cell's postion in the cell cycle, showing a peak of activity in early S phase (Cappechi, page 71, 1st col., 1st parag.)."

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to generate a rat fetal lung cell line comprising a disruption in CIC-2.

One having ordinary skill in the art would have been motivated to generate a fetal rat lung cell line comprising a disruption in CIC-2 in order to obtain a fetal lung cell line that can be used to demonstrate the necessary role of CIC-2 in fetal lung development.

There would have been a reasonable expectation of success given the teachings of Blaisdell et al for demonstrating that KGF upregulates CIC-2 expression in primary cultures of fetal lung cells of rats, that CIC-2 is an ideal candidate chloride channel that has a role in regulating the fluid expansion of the developing lung, and the teachings of Chalaka et at. for demonstrating a fetal rat lung epithelia cell line was made and immortalized with the adenoviral 12S E1A gene product, and Capecchi for demonstrating that homologous recombination is a method that can be used to generate a disruption in a gene of interest.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.".

Only claim 26 was rejected under this heading and claim 26 has been canceled without replacement, thus obviating the rejection.

8. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Simon et al. (1997, Nature Genetics, 17: 171-178), in view of Capecchi (1989, Science, 244: 1288-1292) and Alberts et aI.(1994, Molecular Biology of the Cell, Garland Publishing: New York, 3rd ed.).

The Examiner states, "Simon et al. teach that disruption of the CLCNKB (also known as CIC-Kb) gene in the genome of

humans result in patients exhibiting hypokalaemia, elevated serum bicarbonate levels, salt-wasting, and dehydration (Simon et al., page 175, 2nd col., 1st parag. under "Physiological and Clinical Features," lines 1-12). Simon et al. teach that the mechanism of the defect responsible for the symptoms exhibited by the patients is' a' failure of normal transit of chloride across the basolateteral membrane into the bloodstream (Simon, et at., page 176, 2nd col., 2nd parag., lines 5-7). Simon et at. teach the physiology of sodium chloride reabsorption in the renal thick ascending limb of Henle's loop in the kidney, (Simon et al, Figure 6b).

While Simon et at teach humans comprising a disruption in CIC-1, they do not teach a cell line comprising a disruption in CIC-1.

Capecchi generally teaches a method for performing homologous recombination. In addition to teaching that the method can be applied to generate a mouse comprising a disruption in the gene of interest in the mouse's genome, Capecchi teaches that the method of targeted gene disruption can be applied to cultured cells in vitro. Capecchi teaches that one advantage to carrying out in vitro studies over in vivo studies is that many biological questions can be answered directly and more simply with tissue culture systems (Capecchi, page 1288, 3rd parag., lines 3-4).

The generation of a cell line is well known in the art. For example, Alberts et al. teaches that wile most vertebrate cells die after a finite number of divisions in culture, some cells in culture will undergo a genetic change that makes them effectively immortal. Such cells will proliferate indefinitely and can be propagated as a cell line (Alberts et al., page 160, 1st parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells"). Alberts also teaches that cell lines can be propagated from cancer cells. Cancer cells differ from those prepared from normal cells. For example, cancer cell lines often grown without attaching to a surface and they proliferate to a very much higher density in a culture dish. Similar properties can be experimentally induced in normal cells by transforming them with a tumor-inducing virus or chemical (Alberts et al., page 160, 2nd parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells").

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to generate a cell line obtained from the renal thick ascending limb of a patient comprising a disruption in his/her CLC-NKB gene in his/her genome.

> One having ordinary skill in the art would have been motivated to generate a cell line from the renal thick ascending limb in a human CLC-NKB patient in order to use the renal cell in a study that would elucidate the physiological and biological function of that cell. Further, the renal cell could be used in toxicological and pharmacological drug screening studies.
>
> There would have been a reasonable expectation of success given Simon et al for teaching that CLC-NKB patients exhibit a chloride transport defect in their renal thick ascending limb and Alberts et al. for teaching how to obtain a cell line from a primary culture.
>
> Thus, the claimed invention as a whole was clearly *prima facie* obvious.".

Only claim 26 was rejected under this heading and claim 26 has been canceled without replacement, thus obviating the rejection.

9. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gunther et al., (1998, PNAS, USA, 95: 8075-8080) in view of Capecchi (1989, Science, 24 1288-1292) and Alberts et al.(1994, Molecular Biology of the Cell, Garland Publishing: New York, 3rd ed.).

> The Examiner states, "Gunther et al. teach that CIC-5 provides an electrical shunt that is needed for the efficient proton pumping of the electrogenic H+-ATPase, which is colocalized with CIC-5 (Gunther et at., 1998, PNAS, USA, 95: 8075-8080, Abstract). This idea is supported by the finding that disruption of either the single yeast CLC gene or of a subunit of the V-type H+-ATPase results in a common phenotype (Gunther et al., page 8079, 2nd col., 4th parag., lines 1-6). While Gunther et al. teach that CIC-5 is provides an electrical shunt needed for proton pumping, Gunther et at. do teach a cell line that expresses CIC-5 that could be used to identify and test substances which are suitable for inhibiting CIC-5.
>
> With regards to providing motivation as to why one would want to generate a cell line that expresses CIC-5, Capecchi teaches that one advantage to carrying out in vitro studies over in vivo studies is that many biological questions can be answered directly and more simply with tissue culture systems (Capecchi, page 1288, 3rd parag., lines 3-4). Certainly, using the cells to screen for

compounds that inhibit CIC-5 would be one example of a biological question that could be answered directly and more simply with a tissue culture system.

The generation of a cell line is well known in the art. For example, Alberts et al. . teaches that wile most vertebrate cells die after a finite number of divisions in culture, some cells in culture will undergo a genetic change that makes them effectively immortal. Such cells will proliferate indefinitely and can be propagated as a cell line (Alberts et al., page 160, 1st parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells"). Alberts also teaches that cell lines can be propagated from cancer cells. Cancer cells differ from those prepared from normal cells. For example, cancer cell lines often grown without attaching to a surface and they proliferate to a very much higher density in a culture dish. Similar properties can be experimentally induced in normal cells by transforming them with a tumor-inducing virus or chemical (Alberts et al., page 160, 2nd parag. under "Eucaryotic Cell Lines Are a Widely Used Source of Homogenous Cells").

With regards to expressing CIC-5, the art teaches many ways that CIC-5 can be expressed in a cell line. One way is via transient transfection. Many vectors are available for high levels of expression of exogenous proteins. For example, Invitrogen sells a mammalian expression vector, pcDNA.

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to generate a cell line that expresses CIC-5, in order to identify compounds which inhibit CIC-5.

One having ordinary skill in the art would have been motivated to generate a cell line that expresses CIC-5, in order to use the cell in a method of identifying compounds which inhibit CIC-5.

There would have been a reasonable expectation of success given Piwon et al. and Gunther et al for teaching the role CIC-5 has in interacting with the H+/ATP-ase pump, Cappechi teaching why one would prefer to use an in vitro system over an in vivo system, Alberts for teaching how to make a cell line, and Invitrogen for teaching that pcDNA is a vector that can be used in transient expression of mammalian cells.

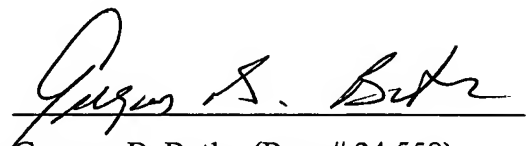
Thus, the claimed invention as a whole was clearly *prima facie* obvious.".

Only claim 38 was rejected under this heading and claim 38 has been canceled, thus obviating the rejection.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. Applicant's representative would like to discuss this case with the Examiner to learn if any outstanding issues remain after consideration of this Amendment. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record. The Applicants believe that a three-month extension of time is required. The Applicants conditionally petition for a further extension of time to provide for the possibility that such a petition has been inadvertently overlooked and is required. As provided below, charge Deposit Account No. **04-1105** for any required fee.

Date: November 18, 2005

Respectfully submitted,



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